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TABLE OF CONTENTS

SECTION V

Evolution of the Seed Habit in Plants. By R. B. Thomson 229

Characters of Common Wheat in Plants with Fourteen Chromosomes. By W. P. Thompson 273

Some Observations on Hydrodictyon reticulatum (L.) Lagerh. With Special Reference to the Chloroplasts and Organization. By Charles W. Lowe 279

Some Freshwater Algae of Southern Quebec. By Charles W. Lowe 291

Sur un Botrychium nouveau de la flore américaine et ses rapports avec le B. Lunaria et le B. simplex. Par Frère Marie-Victorin 319

Cheese Torulae. By F. C. Harrison 341

The Functions of the Cerebellar Nuclei as determined by Faradic Stimulation. By Frederick R. Miller 381

Ayerza's Disease Associated with Primary Carcinoma of the Lung. By William Boyd 383

Peptic Digestion of Coagulated Egg White. By E. Gordon Young and I. G. Macdonald 385

Researches in Craniometry No. 3.—Certain Factors that are Concerned in the Evolution of the Frontal Cranial Arc. By John Cameron 395

Researches in Craniometry No. 4.—A Study of the Chords and Curvatures of the Frontal Cranial Arc in Man and the Anthropoids. By John Cameron 407

Contact Requirements and Reactions. By Arthur Willey..... 421

A Statistical Study of the Head Length Variability of Bovine Spermatozoa and its Application to the Determination of Fertility. By A. Savage, W. W. Williams, and N. M. Fowler 425

The Detailed Anatomy of Triturus Torosus. By Gertrude M. Smith 451

A Bio-Hydrographical Investigation of the Sea Adjacent to the Fraser River Mouth. By C. C. Lucas and A. H. Hutchinson 485

Cheese Torulae

By F. C. HARRISON, F.R.S.C.
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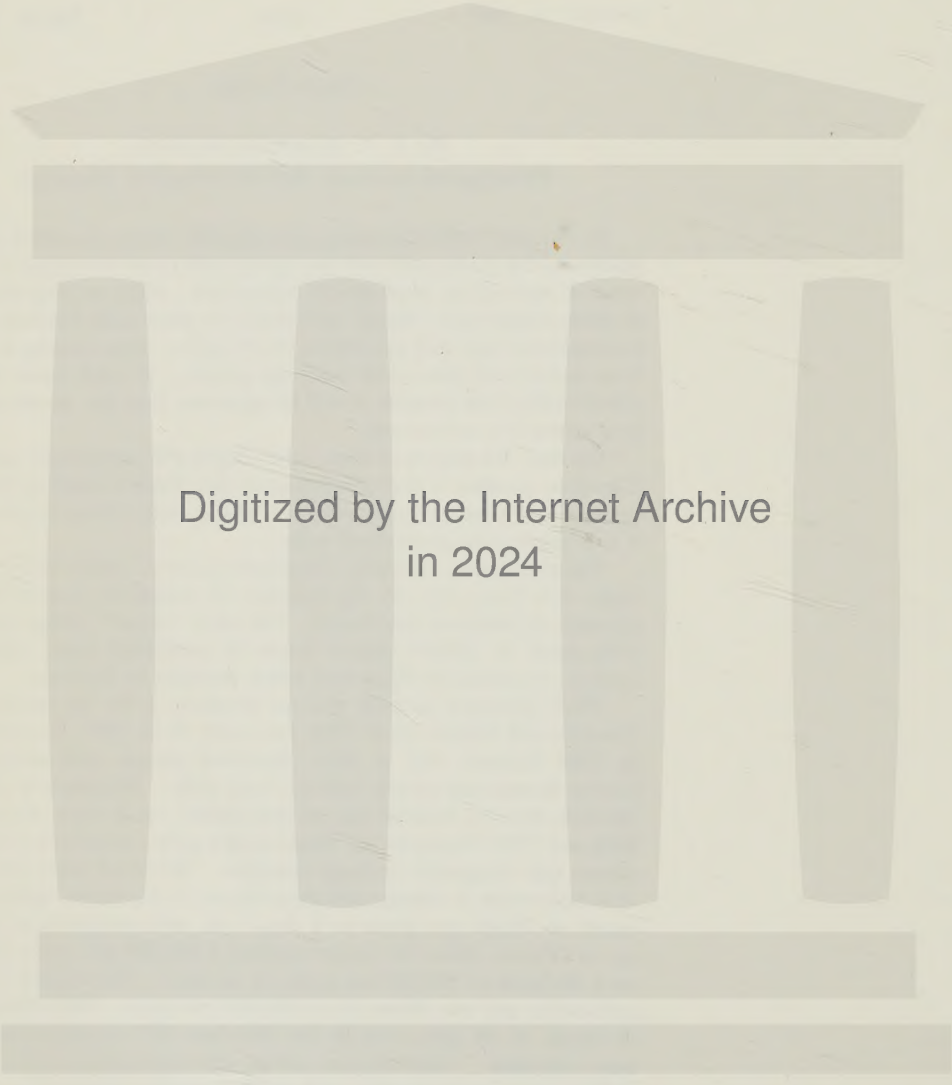
In the year 1926 approximately 216,000 cheese examined by the graders of the Dairy and Cold Storage Branch of the Dominion Department of Agriculture were graded below first. Fifty or sixty per cent. of these cheese were affected with fruity or whey tank flavours and in consequence they were graded as No. 2 quality, thus causing a loss of from one to one and a half cents per pound. As each cheese weighs about eighty-four pounds, it will be apparent that the monetary loss in a season is a serious one.

Further, the export of many such cheese will eventually give the Canadian product a bad reputation in the English market, and our competitors a better opportunity of selling their cheese, particularly if it is made from pasteurized milk.

These flavours, variously described as fruity, sweet, bitter, whey tank, not clean, are, in the majority of instances, caused by the presence of yeasts in the cheese. The term "yeasts" being used in a wide sense to include certain forms of unicellular fungi spherical, oval or cylindrical in shape and which multiply by budding.

Their presence in milk and its products is by no means new, PIROTTA and RIBONI (1) in 1879, DUCLAUX (2) in 1887, ADAMETZ (35) in 1889, KAISER (36) in 1891, described yeasts, and particularly lactose fermenting yeasts isolated from milk. BEIJERINCK (3) and BOCHIOCCHIO (4) isolated lactose fermenting yeasts from cheese. In 1896 and 1897 HARRISON (5) found yeasts quite common in Canadian cheese and frequently in large numbers. He noted that they were able to increase in cheese, and gives figures of one cheese with a yeast count of 10,000 per gram at 2 days old, with progressive increase up to 23 days, when the count reached 1,500,000 per gram followed by a decrease to 500,000 per gram at 45 days. The largest number reported in any one cheese was 6,000,000 per gram. They were found in cheese of all ages, even in one 403 days old, 50 yeasts per gram were obtained. Their diverse habits were mentioned, such as their ability to grow in acid whey and produce bad flavours and mottles in coloured cheese when associated with lactic acid bacteria. Subsequently in 1900 HARRISON (5) described and named a yeast (*Torula amara*) producing a bitter, astringent flavour in cheese. Harding (6) described a sweet or fruity flavour in cheese in New York State due



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to yeasts, but did not describe them. HASTINGS (7) in 1905 and 1906 reported a widespread distribution of lactose fermenting yeasts in Wisconsin but did not consider them of much economic importance to the dairy industry. EDWARDS (8) in 1913 isolated a number of yeasts from fruity or sweet flavoured cheese produced by factories in Western Ontario. Experimental cheese made up with various cultures of these yeasts were all of bad flavour —some being fruity, others bitter, yeasty, dirty or rancid.

CORDES and HAMMER (9) pointed out that yeasts were responsible for gas production in sweetened condensed milk, and that they remained alive a long time in butter even after the bacteria had died. They suggested a grouping into lactose and saccharose fermenters, production of pink colour, and digestion of milk and in a later paper suggested a further grouping according to colour production, colony formation, and production of gas in sucrose media.

The species described in milk and its products have been reviewed in some of the publications mentioned and also by GUILLIERMOND.(10). A large number of other Torulae obtained in the brewing and wine industries have been described with a few exceptions, by number. For example, WILL (11) isolated seventeen forms from the air in breweries, etc., and a number of other authors have given such meagre descriptions that it is impossible to identify them.

The difficulty of adequate description is no doubt quite complex. LEBERLE & WILL (12) have suggested that characteristics for the differentiation of the Mycoderma and Torula should include biochemical reactions and resistance of the varieties towards alcohol, etc. LUTZ and GUEGEN (13) have proposed the use of certain media, as RAULIN's solution with and without sugars, and various other media. Neither of these methods has been used sufficiently to ascertain their value but all investigators agree as to the difficulty of characterizing the asporogenous yeasts.

This investigation was undertaken in order to obtain:

1. Information regarding the number and kind of yeasts present in Canadian cheese.
2. To ascertain if they produce the various kinds of off flavours noted by the grading experts, to which different names, such as bitter, fruity, off-flavour, not clean, whey tank, etc., are applied.
3. To attempt to systematize the species and varieties so that they may be recognized at any time.
4. To find out their habitat and how they gain access to the milk.

5. To study their viability and life history in order to obtain all necessary information to give to the cheesemaker, in order that he can control or prevent their growth in milk and its products.

The samples of cheese examined were obtained from warehouses in Montreal, and were sent to me by MR. J. BURGESS, Chief, Division of Dairy Products of the Dairy & Cold Storage Branch of the Dominion Department of Agriculture, whose co-operation, and that of his staff, have been most helpful. Each sample consisting of several plugs obtained with a cheese trier was placed in a sterile jar, and sent direct to the laboratory, together with particulars regarding factory, vat number, and defect as judged by the official graders. From DR. E. G. HOOD of the Dairy & Cold Storage Branch, Ottawa, I received a number of plates prepared from cheese coming from Dundas County Ontario, which contained numerous yeasts. In all, over a hundred samples of Canadian cheese have been examined from factories well scattered over Ontario and Quebec, and, in addition, two samples of cheese from New Zealand.

METHODS OF EXAMINATION.

All cheese have been examined microscopically by means of impression preparations, stained by GRAM'S method, and counter stained with dilute saffranin. The bacteriological analyses were made by the method already described by HARRISON (5). For isolation of yeasts beer wort agar pH 4.6 was used. The plates were poured, allowed to set, and from the dilution in sterilized water a small portion was brushed over the surface with sterilized brushes, or about five cc. of the dilution was poured over the agar and allowed to drain off. This method favours yeast growth and gives better differentiation than plates made in the ordinary manner.

The yeast colonies were picked off in the usual manner, sub-cultured in wort agar, transferred to beer wort, and from this medium single cell isolations were made by the method of HANSEN in wort gelatine, again transferred to wort agar from which all subsequent transfers were made. Thus all cultures were transferred five times before cultures were examined, stained by GRAM'S method and living preparations made from at least five different culture media.

Films or scums were examined for possible spore formation, and young wort cultures 48 hours old, were transferred to plaster blocks, kept at 20 to 25 and 37° C., and examined at intervals for spores.

Culture Media

The media employed, unless otherwise noted, were made according to the suggestions of the committee on bacteriological technique of the Society of American Bacteriologists. Indicators used were: Andrade, Brom cresol purple and Brom phenol blue. All cultures were observed daily for fourteen days; observations on many were extended to seventy days. Beer wort 11.2 Ball. was used, diluted with an equal quantity of water: reaction pH 6.6. Agar and gelatine were made from this wort, the former adjusted to pH 4.6 and an indicator brom phenol blue added. Silver nitrate wort agar was made by the addition of 1 c.c. of a 5 per cent. solution of silver nitrate added to each 100 c.c. of wort agar. All the cultures were grown on corn meal agar (Difco), but it was not a good medium for growth or differentiation. The same was found true for sliced carrot or potato. The sodium citrate medium was made with 1 per cent. medium citrate in yeast water broth reaction pH 7.0.

The sugars used were of the highest purity, either Merck's or Difco's. The selection was made to include the aldohexoses; dextrose, mannose, galactose the ketohexose fructose; the pentoses, arabinose and xylose; the methyl pentose rhamnose, the di-saccharides maltose, lactose and saccharose; the trisaccharide raffinose; the polysaccharides, inulin and dextrin, and in some instances soluble starch; the alcohols, glycerine, mannite, sorbite, and dulcite; the glucoside salicin, and the cycloparafin, Inosite (all D. forms except fructose).

One or two per cent. of the above carbohydrates were used in Dunham's broth. In the text these media are referred to by the name of the carbohydrate.

Other media tried out extensively on at least thirty different species were Laurent's medium and Cohn's solution, with the addition of sugars; as they did not give as good results as peptone broth they were abandoned.

The raisin extract was made from 10 per cent. of raisins and one per cent. of peptone, with the addition of alcohol after sterilization to make a 5 per cent. solution. The pH was 7.0 and brom phenol blue added to one lot and brom cresol purple to another.

Wort agar, with the addition of brom phenol blue, made an interesting medium. The original pH was 6.0. The range of the indicator 3.0 to 4.6. On this medium a variety of colours were produced by the organisms. Some remained white, others absorbed the colour in varying degree. The accumulation of dyes by living cells

has been the subject of a number of investigations (15), and several theories. As a number of these torulae do not absorb the dye from the media, and others do, in varying degrees, it seems to show that the accumulation of dye is the result of a work done by living protoplasm. Difference of pH does not seem to influence the penetration of dye, as the substratum in some cases in which the cells were coloured was quite low (3.0) and in other cases more than 4.6.

Impression Preparations

From each cheese three to six impression preparations were made, each time using a fresh cut surface from two or more plugs. These preparations gave a very good idea of the bacteria and yeast content, and the ratio of one organism to the others. By an adaptation of Hucker's suggestion the total yeast content was computed direct.

As yeasts are relatively large, a high dry objective and high ocular may be used instead of an oil immersion lens, giving a larger field. It was assumed that the film made by impression was 1 μ in thickness, and using the formula suggested by Hucker, the amount of cheese examined per field would be approximately $\frac{1}{18{,}000{,}000}$ gram. That is, each yeast cell observed in a single field represents 18,000,000 per gram. The difficulty of approximating the exact number is largely due to the clumping of the cells, and this error can be only partly corrected by counting a large number of fields. For example, in 100 fields 90 were free, and the remaining 10 were as follows: 1, 5, 1, 1, 3, 15, 43, 3, 5, 1 = 78; therefore yeast content was in round numbers 13,000,000 per gram. Compared with the plate counts in wort agar the direct count was invariably higher, a few examples will show the relation.

Direct counts of yeasts compared with plate counts:

Cheese	Direct count	Plate count
278 fresh	23,000,000	9,500,000
571 fresh	2,000,000	700,000
335 fresh	8,000,000	2,500,000
893 old	17,000,000	7,500,000
636 fresh	500,000	100,000

Undoubtedly the method gives better results with yeast than with bacteria, owing to the larger size of the yeasts, and the impression preparation having much less depth than the sections little or no adjustment of focus is necessary.

In all cases an approximation of the number of yeasts per gram

by the direct count was made; Table I gives a selection of these counts together with the remarks of the grader.

Cheese Factory	Grader's remarks	Yeasts by direct count
348 (old)	Not clean	13,000,000
1631 (old)	Very unclean	7,000,000
431 (fresh)	Fruity	31,000,000
2214 (fresh)	Fruity	19,000,000
276 (fresh)	Fruity	3,500,000
636 (fresh)	Good	50,000
246 (fresh)	Not clean	11,000,000
21 (fresh)	Fruity	4,500,000
615 (fresh)	Not clean, whey tank, weak	43,000,000
571 (fresh)	Not clean, old milk	17,500,000

Dr. E. G. Hood, of the Dairy and Cold Storage Branch, Ottawa, has kindly furnished me with counts of yeasts from 37 cheese factories in Dundas Co. Wort agar, pH 3.6, medium was used. The lowest number was 1,400 the highest 1,800,000, and the average of the 37 - 200,000 per gram.

As with bacteria, so also with yeasts, in cheese there is a tendency to form clumps or colonies. In some cases as many as 300 yeast cells have been counted in a single field (Figs. 1 and 2). Aggregations of three and four up to twenty and thirty are common, but single individuals also occur. Gram positive cocci, streptococci and bacilli were noted; also gram negative organisms. A protocol of a sample of cheese taken from my notes shows the information gained by the impression preparation:

Cheese: Vat No. 248, Factory 348. Defect: Not clean. Age: Old.

Impression prep. Gram, saffranin. 2 plugs. 4 preparations.

Gram Positive:

1. *Yeasts Torula.* Numerous medium sized, ellipsoidal torulae. Direct count 13,000,000 per gram; occur singly and in large and small colonies.
2. *Streptococcus lactis.* In pairs very numerous, singly and in colonies.
3. *Large bacillus.* Rounded ends, numerous, often in colonies.
4. *Short, stout bacullis.* Rounded end, occasional.

Gram Negative:

1. A *large bacillus* with rounded ends, a few.
2. A *small, thin bacillus*, rare, 3 times as long as wide.

Wort agar plates, isolations, etc.

From a study of these preparations, made as stated from about one hundred samples of cheese, the following facts seem well substantiated:

1. Cheese graded as fruity, not clean, whey tank, etc., contained large numbers of yeasts. (As yeast cells are so much larger than bacterial cells, it is reasonable to suppose that they produce enzymes and products commensurate with their size hence a million yeast cells per gram would be at least equivalent to ten times the number of *S. lactis*.) 2. Cheese graded as good contained yeast cells but in much smaller numbers. 3. All Canadian cheese examined contained yeast cells. 4. The yeast cells occurred singly, in small and large groups or clumps (see Figs. 1 and 2). 5. Two samples of New Zealand cheese contained no yeast cells (microscopic and cultural methods). 6. Many GRAM positive and negative bacteria were observed but comment regarding these is omitted as this investigation was primarily concerned with yeasts.

The only true saccharomycete isolated from cheese belongs to GUILLIERMOND'S (10) second sub-group of the genus *Saccharomyces*. It is almost intermediate between the first and second groups as it ferments dextrose and saccharose and produces acid but no gas in maltose. It was isolated from three samples of cheese coming from widely separated factories.

Saccharomyces casei Harrison. The cells are usually ellipsoidal 4.8×3.0 μ to 7.6×4.8 μ. Round cells also occur averaging 4.8 μ in diameter. From a wort film 70 days old, ellipsoidal and round forms occur approximately the same size as mentioned above (Fig. 3).

Spores are present in the old films. On plaster blocks spores appear in about 30 hours at 20° C., but no spore formation was noticed at 37° C. (9 days). The spores are two to four in number, usually four, and average 2.8 μ in diameter. They germinate by budding. The spores swell, become angular at intersecting points, and finally break through the wall of the asc and start budding. Figs. 4 and 5 show the ruptured wall of the asc, the swollen spores and the spores in the asc.

In wort a ring is first formed, later an islet film which is easily disturbed. The wort becomes clear, with a heavy sediment. In wort agar there is a dull, white, moist, glistening growth, becoming duller in four days at 22° C.

In dextrose with peptone or LAURENT'S medium, mannose, d-galactose and fructose, acid and gas are formed. pH 5.2 in mannose.

On wort agar with brom-phenol blue, the growth is white with a

faint blue stripe down the centre; pH of medium after two weeks growth 4.0.

It produces acid and gas in saccharose, acid but no gas in maltose, slight acid but no gas in dextrin, and no acid or gas in arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, lactose raffinose, salicin, inulin, and soluble starch. In yeast water citrate it forms acid, pH 5.4.

In milk it grows well, producing a copious sediment, but no coagulation or other change; the odour is unpleasant and yeasty. In wort gelatine stick culture there is a coarsely beaded, confluent growth, with gas bubbles. Later (37 days) small villous tufts grow from the beaded growth. No liquefaction in 40 days.

The giant colony on wort gelatine exhibits a scalloped margin, cream white centre, concentrically contoured, with radiate markings.

It grows well at 20-22 and also at 37° C. Thermal death point 65° C. for ten minutes.

According to GUILLIERMOND (10), the genus *Torula* is characterized as follows:

"Generally spherical cells, often forming a scum but only after fermentation, scum always slimy without the presence of air bubbles. He also states that they contain a globule of fat which gives them a special characteristic. Some are oval or elongated and offer the appearance of ordinary yeasts. A certain number form pigments."

Originally the name Torula was given to hyphomycetes with round or oval chains (16). TURPIN in 1838 called *Sacch. cereviseae*, *Torula cereviseae*.

Cohn applied the name to chains of cells formed by the genus *Micrococcus*. PASTEUR (17) used the name for yeast fungi producing small quantities of alcohol, but does not mention whether they formed spores or not. HANSEN (18) regarded Torulae as yeast cells similar to the Saccharomyces but which did not form endospores nor develop typical mould growths. As regards alcohol production, they may exhibit this in all degrees.

Yeast nomenclature is almost in as chaotic a condition as bacterial nomenclature. In characterizing the genus *Torula* we have a mixture of morphological and physiological characters. To adhere to one or both is likely to provoke criticism. Any system of classification which will permit easy identification is, however, of manifest advantage, and as our knowledge of these fungi increases, there will be necessary revision.

The first Torula discovered seems to be the organism described by FRESENIUS (19) in 1850, who isolated a red yeast from dust of

the air and named it *Crypto-coccus glutinis*. (The genus *Cryptococcus* has been pre-empted by VUILLEMIN for pathogenic yeasts which do not sporulate. A decided protest has been made by some bacteriologists against classification by habitat. There seems no valid objection to placing these yeasts in the genus *Torula*.) Later it was isolated by COHN (20) and SCHRÖTER. HANSEN found that the two species described by the above authors did not correspond. HANSEN described others, and later PRINGSHEIM and BILEWSKY (22) isolated another red yeast which resembled *Cryptococcus glutinis* of FRESENIUS and named it *Torula glutinis*.

The genus *Torula*, however, was named by TURPIN in 1838, who called the yeast now known as *Saccharomyces cerevisiae*, *Torula cerevisiae*. WILL (23), in his historical account of the *Torula*, commences with PASTEUR in 1862. Usage undoubtedly favours the name *Torula*, but it is difficult to characterize the type species. I suggest, therefore, that the genus *Torula* be changed to a family with the designation Torulaceae with three genera: *Rhodotorula*, *Chromotorula* and *Torula*. German investigators frequently use the term Torulaceen.

Family Torulaceae: Unicellular fungi, spherical, ellipsoidal or cylindrical. Do not produce spores; often contain globules of fat. A certain number produce pigments: red, yellow, brown or black. Some form a ring, scum, or pellicle or film without the presence of air bubbles. Others do not. Often capable of fermenting sugars.

Genus 1. *Rhodotorula* Harrison. The characters of the family, producing pink or red pigment. In this genus would be included all the red yeasts of medical writers, and the following species: *T. pulcherrima* Lindner (24); *T. rubefaciens* Grosbusch (25); *T. mucilaginosa* Jorgensen (26); *T. roseaca* Van Hest (27); *T. glutinis* Sartory (28), etc., etc., these would become *Rhodotorula pulcherrima*, etc. The type species *Rhodotorula glutinis* Harrison, syn. *Cryptococcus glutinis* Fresenius, *Sacc. glutinis* Cohn, *Torula glutinis* Pringsheim & Bilewsky –oval cells, average dimension 5 to 11 $\mu \times 4 \mu$. Optimum temperature for budding is between 22 and 30° C.; at 37 to 38° C. the yeast stops budding.

On glycerol broth it forms a scum made up of cells in the form of mycelium. Oval cells in the sediment. On carrot produces a red layer; also on potato and artichoke, but less rapidly. On gelatin and agar forms small red colonies. Liquefies gelatine after a certain time. Without action on d. galactose, maltose, starch and inulin. In milk in 14 days it precipitates the casein with no digestion.

Genus 2. *Chromotorula* Harrison. The characters of the family, producing yellow, brown or black pigment.

Type—*Chromotorula Kitae* Harrison (Syn. *Torula* of KITA (29).

Cells usually round, sometimes ellipsoidal with thick walls, vacuoles seldom seen, size of cells 4.5 to 8 μ. Produces round or star-shaped yellow colonies, elevated in the middle with a smooth periphery. On "Koji" extract gelatine and agar. Giant colonies yellow with sunken centre, granular surface and wavy periphery. Streak cultures moist, yellow, granular with wavy edges. Grows in 10 per cent. salt solution of "Koji" extract producing flocculence with the formation of a ring. It ferments glucose, maltose, but not galactose, sucrose, lactose, raffinose nor arabinose. Optimum temperature about 28° C.

Other Torulae belonging to this group would be the orange yeast of KALANTHAR (30). The black yeasts of MARPMANN (31), WILL (32) and other are probably allied to the genus *Dematium*.

Genus 3. *Torula*.

The characteristics of the family but producing no pigment. There is much difficulty in selecting the type species. PASTEUR'S *Torula* isolated from beer are insufficiently described. HANSEN described a number of Torulae by number. Other authors have insufficiently described species, or have used numbers. The first good description of a Torula is that given by DUCLAUX (2), hence this yeast is selected as the type species.

Torula Duclauxii Harrison (Syn. DUCLAUX'S (2) *Torula*).

I have divided this genus into subgroups. GUILLIERMOND (10) has used this method in describing his genius IX, *Saccharomyces*:

Group A—No acid or gas in any sugar.

" B—Slight acid in dextrose, mannose, fructose or galactose.

" C—Slight acid with or without trace of gas in dextrose, mannose, fructose or galactose and saccharose.

" D—Marked acidity and gas in dextrose, mannose, fructose or galactose.

" E—Marked acidity and gas in dextrose, mannose, fructose, galactose and saccharose.

" F—Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and raffinose.

" G—Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and maltose.

" H—Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and lactose.

" I—Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose, lactose and inulin.

This may seem a large number of subgroups, but after an experience of attempting to characterize several hundred Torulae isolated from cheese I have found this outline of great service for the purposes of classification. The grouping is suitable for Torulae isolated from other sources, but it may be necessary to add a few more groups for yeasts with greater diversity of fermentative activity than those described here. As this investigation concerns the yeasts isolated from cheese, I record in this suggested classification the Torulae I have isolated.

Rhodotorula corallina Harrison (Fig. 6)

Red Torulae have been frequently isolated from cheese. As a rule they are in small numbers, but occasionally they are found in such numbers that their presence is indicative of massive infection, or of considerable growth in milk or cheese.

The following species were occasionally isolated: *Torula rubefaciens* (Grosbusch) and the Torula described as *C. glutinis* by SARTORY, which is different from the one originally described under that name, and which I suggest should be called *Rhodotorula Sartoryii* Harrison.

A red Torula found in large numbers in two cheese, 50,000 to 75,000 per gram, which seems to be undescribed I have named *Rhodotorula corallina* on account of its coral red colour. *Rhodotorula corallina* Harrison (Fig. 6).

Cells round or ellipsoidal, the former average 3.6 μ and the latter 4.0×2.0 μ. From a ring formed in wort after 70 days growth the cells were round and very even in size, 3.6 μ.

In wort it grows well, producing turbidity and a heavy sediment, a pink ring is first formed, then an islet film, pink in colour. The sediment is pink.

In wort agar at 22° C. it grows quickly, producing a pink, moist, glistening, massive growth. On brom-phenol-blue wort agar the growth is similar, final pH of medium 4.6. With the addition of a few drops of silver nitrate (5 per cent. sol.) to the agar the colour is slightly intensified. Coral red best describes the colour.

In dextrose it produces marked acidity but no gas or alcohol. In fructose faint acidity and no gas. In mannose, galactose, arabinose, inosite, maltose, lactose, saccharose, raffinose, salicin, inulin, dextrin, and soluble starch it grows well, producing a ring or scum, pink in colour; in some of the above turbidity, in others clear. In raisin extract with 5 per cent. alcohol it grows well, producing a pink film and ring and acidity pH 5.2.

In litmus milk there is no apparent change other than a pink

sediment for 30 days. At 40 days there is a gelatinous coagulation, the upper third is blue, middle third reddish, and lower third white, with a pink sediment.

In gelatin stick culture there is a filiform growth, pink in the upper third and a raised pink surface growth, which gradually sinks, slowly liquefying the gelatine. In 37 days about one and one-half cm. of gelatine are liquefied.

The giant colony in wort gelatine is round, raised and coral pink in colour. Liquefaction begins in 8 days and is more rapid than in tubes.

Pigment not soluble in water, alcohol, acetone and ether. Grows well at all temperatures between 20 and 30. At 37° C. growth is slower. Thermal death point 60° C. for 10 minutes.

Chromotorula ochracea Harrison (Fig. 7).

This Torula was isolated from three different cheese from widely separated localities. Dr. E. G. Hood sent me two cultures of the same species from two different factories. He thought there were slight differences in colour, but on culture there were no marked variations.

The cells are ellipsoidal to cylindrical, and form at times a mycelial growth. The ellipsoidal cells range from $5\times3\ \mu$ to $12\times8\ \mu$. Pigment in cells is soluble and appears olive green in colour and located in the vacuole and oil drops. There are numerous fat globules in older cells which are often in a circle around one end of the vacuole. The oil globules coalesce when placed in melted gelatine. Budding from the ends or shoulders or both, the buds form close to the mother cell or may have a connecting tubule often $2\ \mu$ long (see Fig. 7). The cells from the film in wort (70 days are ellipsoidal and many long and cylindrical. From the sediment the cells are less pigmented and ellipsoidal forms predominate; round cells also frequent. From silver nitrate agar the cells are ellipsoidal, averaging $9.6\times6.0\ \mu$.

In wort a partial film forms in 24 hours, liquid clear and heavy sediment. In 7 days film and sediment very marked. The film gradually becomes yellow (25 to 30 days) then greenish black (54 days). The film is tough but not mucilaginous.

On wort gelatin plates the colonies appear as sulphur yellow to ochre depending on age, often pulvinate and rugose. On wort agar the growth is at first whitish, dull and rugose to vermiform, in four days it becomes pale yellow, then ochre to dark green to green black; the final change taking from 20 to 30 days or longer from the time of transfer. Agar with brom-phenol-blue gives similar growth. The pH

of medium in two weeks is 4.6. On silver nitrate agar the growth for three weeks is white but changes to a dirty brown.

In dextrose and fructoes it produces acid and gas, 50 to 100 per cent. of closed arm. In galactose and mannose, acid but no gas. pH 5.2. In saccharose, acid and a trace of gas (½ per cent.).

There is good growth in the following, but no acid, gas, film, nor ring: arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, lactose, raffinose, salicin, inulin, dextrin, soluble starch, whey (film), yeast water citrate (film). In raisin extract with 5 per cent. alcohol it grows producing a yellow film.

In milk there is no apparent change for seven days, then a yellow ring forms, and in fifty days there is a soft curd with no separation of whey, the upper portion gradually turns greenish, the lower remains white. The odour in young milk cultures is yeasty. (Grader's remarks.)

In gelatin stick culture there is a papillate growth and infundibuliform liquefaction complete in 18 days. The pellicle on the surface is at first cream colour, then yellow and finally greenish black.

The giant colony on wort gelatine is large and has alternate zones of yellow and white. It liquefies in five days.

It grows well at temperatures between 20-30. At 37° C. it gives scant growth. Thermal death point 60° C. for 10 minutes.

Genus Torula.

Group A. No acid or gas in dextrose, mannose, galactose and fructose.

Numerous Torulae have been isolated from cheese which have no action on the simple carbohydrates and higher alcohols. There are small differences in morphology and cultures, hardly sufficient, however, to warrant naming them all as new species. The type for the group is *Torula dubia* Harrison (Fig. 8).

The cells are usually ellipsoidal, from 2.5×2.0 to 4.5×3.0 μ; average 3.6×2.4, budding from ends or shoulders, buds often connected to mother cell by a small tubule. From the film on wort after 50 days at room temperature the cells are frequently round and very even in size, averaging 3.2 μ in diameter. Ellipsoidal cells are present and a few cylindrical uneven in diameter, curved, or thicker at one, both ends, or middle.

In wort there is cloudiness, and a ring is formed in 7 days and a film in 14 days. This film is easily disturbed. The liquid clears and a heavy sediment is formed.

On wort agar there is a whitish spreading, moist growth which becomes duller (4 days) and slightly contoured (15 days). On agar with brom-phenol-blue indicator the growth is white, a pale blue streak develops in the lower part of the slope and the pH of the medium is 4.6 after 14 days' growth.

In the following media there is good growth, at first cloudy, then agglutinating and later clearing with slight or copious sediment.

No acid or gas is formed in dextrose, mannose, galactose, fructose, arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, lactose, saccharose, raffinose, salicin, inulin, dextrin, soluble starch, whey and yeast-water citrate. On raisin extract with 5 per cent. alcohol there is good growth and the formation of first a ring, then a film. The ring is blue from the indicator B.P.B., but there is no change in pH of the medium. In milk there is good growth, but no apparent change; a whitish sediment forms. The growth in wort gelatine (stick culture) is at first finely beaded. In five days the upper third becomes villous, in twelve days villous to arborescent. Surface growth has concentric rings. No liquefaction (50 days).

Giant colony in wort gelatine in 10 days at room temperature is white to porcelain white, entire or slightly undulate edge, elevated centre; not characteristic.

Optimum for growth 22-30° C. Slight growth at 37° C. Thermal death point 60° for ten minutes.

A chemical examination was made of the products produced by this organism. It was grown for 21 days in 500 cc. of glucose peptone broth at room temperature, and then analyzed according to the outline suggested by CHESTER (33) with supplementary tests as given by ALLAN (34) and DUCLAUX (37). It did not produce alcohol, aldehyde or acetone. The first distillate of 50 cc. had a somewhat unpleasant, pronounced odour.

No acids were detected, the initial acidity of the whole 500 cc. was only .5 cc. of normal sodium hydrate. Tests for acetic, lactic, succinic, butyric and formic acids were all negative.

On account of its negative, indefinite physiology, I have named it *dubia*. Closely allied to this species are isolations numbered 34, 40, 51, 67, 42 in the large table. These differ from *Torula dubia* as follows:

(*a*) Cells somewhat rounder at ends. On B.P.B. agar there is a blue streak with white margin final pH 4.4 (Fig. 9).

(*b*) No colour on B.P.B. agar. pH 4.0. Growth on wort agar flat and dull. No contouring. A film forms on saccharose (Fig. 10).

(*c*) Forms a film on galactose, levulose, glycerine, mannite, saccharose, inulin, dextrin, and a ring in Y.W. citrate, whey and milk (Fig. 11). Many cells with ends slightly pointed.

(*d*) Forms a film on whey, grows well at 37° C. (Fig. 12).

(*e*) Gives a faint blue growth in B.P.B. wort agar. pH of medium 4.0. Forms acid, but no gas in saccharose, and a ring and islet film (Fig. 13).

Group B. Slight acid in dextrose, mannose, galactose or fructose.

Torulae in this group give slight acidity in one, two, three or four of the above mentioned sugars. It is possible that slight amounts of gas form, but the CO_2 is at once absorbed by the liquid, and although observations were made daily, no gas was observed in the closed tube. This supposition is verified by the fact that a small amount of alcohol was detected in a 500 c.c. culture. The amount of acid formed is small. Tested with Andrade indicator a faint pink or pink colour develops. With brom cresol purple, pH is around 5.8.

Members of this group have been frequently isolated from many different cheese. The morphology and cultural characteristics of twelve species or varieties are recorded. In classifying them, their morphological differences have been emphasized (Figs. 14 to 24).

Torula subacida Harrison (Fig. 14).

Cells are round or slightly ellipsoidal, round cells average 3.4 μ. Ellipsoidal 3.5 $\times$ 2.2 μ, budding from ends or shoulders. From the film and ring from wort, 70 days at room temperature, round or oval cells predominate, some cylindrical cells forming from round cells and *vice versa*, some cylindrical cells 9.6$\times$3.0 μ.

On wort there is good growth with film formation, cloudy liquid and sediment. In 30 days the liquid clears and remains clear. The film persists 70 days, also a marked ring.

On wort agar there is a flat, spreading, whitish growth, moist at first, but becoming more dull.

On agar with B.P.B. there is a moist, shiny, spreading growth; later a faint trace of blue which turns white. pH of medium after two weeks, 4.6.

In dextrose, slight acidity develops in four days; liquid turbid, but no gas.

In mannose, galactose and fructose there is cloudiness which clears in 7-10 days, copious sediment, but no acidity or gas.

It grows well in the following sugars, but gives no acidity or gas: arabinose, xylose, rhamnose, glycerine, mannite, dulcite, inosite,

maltose, lactose, saccharose, raffinose, salicin, inulin, dextrine, soluble starch and whey. In raisin extract with 5 per cent. alcohol there is a film blue in colour from absorption of the indicator (B.P.B.).

In milk there is no apparent change, odour unpleasant.

Stick culture in wort gelatine. Filiform to papillate along the stick. Surface growth, dull and saucer-shaped, gradually sinking into gelatine. No liquefaction (50 days).

Giant colony, large dull white colour, edges lobate and scalloped. Contoured, rugose, and radial markings (21 days). Grows best between 20-30° C. Grows at 37° C., but not luxuriantly. Thermal death point 60° C. for 10 minutes.

Torula acidula Harrison (Fig. 15).

Cells ellipsoidal from 2.0×1.0 μ to 4.0×2.0 μ, average 2.5×1.5 μ. From wort film 70 days old cells are round 2.0 to 6.4 μ. The large ones with very large vacuole, ellipsoidal from 3.1×1.6 to 5.0×2.5 μ and cylindrical 4.0×1.6 to 10.0×1.6 μ. There is much variation in the appearance of the ends, some round, others pointed, truncate, etc.

In wort it produces a ring and film with cloudy liquid and sediment.

On wort agar there is a moist, white, raised shiny growth, with a ridge down the centre.

On B.P. agar there is a moist, white shiny growth. pH of medium after 14 days' growth, 4.6.

In dextrose the reaction is slightly acid in four days, also in mannose, galactose and fructose, most marked in fructose. No acid or gas in arabinose, xylose, rhamnose, glycerine, mannite, dulcite, maltose, lactose, saccharose, raffinose, salicin, inulin, dextrin, soluble starch and yeast water citrate.

In raisin extract with 5 per cent. alcohol a ring forms.

Milk becomes very slightly alkaline (30 days), but no other change.

In wort gelatine a villous to filamentous growth along the line of puncture, tapering from long hairlike outgrowths near surface to shorter ones half way down the tube. Surface growth, white, raised and slightly contoured, later sinking into the gelatine but without liquefaction (50 days).

Giant colony, large, white, dull, raised centre, faint radiate markings towards edge. No liquefaction (28 days).

Grows well at room temperature 20-25° C., but scantily at 37° C. Thermal death point 60° C. for ten minutes.

The chemical analysis of 500 cc. of dextrose, peptone broth inoculated with this organism and grown at room temperature for 23

days was as follows: 1.5 cc. of normal sodium hydrate required to neutralize the 500 cc.; a trace of alcohol was found (*iodoform* test), no acetone and no aldehyde; the first distillate had an unpleasant odour; tests for lactic, formic and butyric acids all negative; slight trace of acetic acid.

Allied to this species (*Tortula acidula*) being morphologically somewhat similar, but on the whole somewhat larger in size, and differing slightly in fermentative activity, film and ring formation are the following:

Var. (*a*) Gives no acidity in galactose (Fig. 16).

" (*b*) Gives a striped growth on B.P.B. agar; produces no acid in galactose and forms a ring in glycerine and dextrin (Fig. 17).

" (*c*) Gives a striped growth on B.P.B. agar, produces no acid in mannose (Fig. 18).

" (*d*) Gives a deep cerulean blue on B.P.B. agar; produces no acid in mannose and galactose, and forms a film on all four hexoses and a film or ring in glycerine, mannite, inosite, maltose and whey (Fig. 19).

" (*e*) Gives a deep cerulean blue on B.P.B. agar; produces no acid in mannose and galactose, and forms a film on the four hexoses, glycerine, mannite, sorbite, lactose and whey (Fig. 20).

" (*f*) Gives a greenish white growth on B.P.B. agar; produces no acid in mannose and galactose, forms a film on fructose, and the giant colony is round with a concave centre and raised margin (Fig. 21).

The hexose fermentations (acid) and film or ring formation of this group:

Name or No.	Dextrose	Mannose	Galactose	Fructose
Torula acidula	+	+F	+R	+
Var. a	+	+F	−	+
" *b*	+	+	−	+F
" *c*	+	−R	R	+R
" *d*	+F	−F	−F	+F
" *e*	+F	−F	−F	+F
" *f*	+	−F	−F	+

Torula angusta Harrison.

Cells ellipsoidal to cylindrical, much variation of size and shape (see Fig. 22), 5.0×3.0 μ to 2.5×0.8 μ rounded or somewhat pointed ends: general appearance of cells cylindrical. Cells from wort film,

70 days old, mostly ellipsoidal 2.8×1.2 μ with rounded ends. Budding from ends or shoulders.

In wort it forms a film, liquid cloudy at first, then agglutinating and clearing, sediment copious.

On wort agar a moist, white, spreading, shiny growth forms, becoming more massive and duller with age. On B.P.B. agar growth is white to cream colour. pH of medium after two weeks, 4.6.

In dextrose, mannose and fructose it produces acid but no gas. Acidity greatest in fructose, in mannose pH is 5.2. In galactose, arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, lactose, saccharose, raffinose, salicin, inulin, dextrin, whey and sodium citrate, no acid or gas forms. Film formation on fructose. It grows in raisin extract with 5 per cent. alcohol, producing a ring. In milk there is no change, odour unpleasant, yeasty.

Growth in wort gelatine stick culture, filiform. Surface growth gradually sinks, but no liquefaction.

Giant colony on wort gelatine, large, flat, dull and spreading.

Grows well at room temperature and at 37° C. Thermal death point 60° C. for ten minutes.

Torula rostella Harrison.

Cells large, ellipsoidal, cylindrical and irregular, budding chiefly at ends, but also at sides and shoulders. Cells are frequently pointed at one or both ends, due to the large tubule or beak between mother cell and buds (see Fig. 23). Size varies. Ellipsoidal cells 6.0×2.5 μ. Cylindrical cells all sizes some as large as 12.0×3.0 μ. From the wort film, 70 days old, the cells were of all shapes and sizes, many bizarre shapes. The ellipsoidal cells were often large 9.0×4.5 μ.

In wort a film forms which is easily disturbed and sinks, clouding the liquid; sediment very copious, liquid clears but becomes cloudy with slight disturbance of film.

On wort agar there is a moist, white, glistening growth, which later (8 days) becomes heavier and of a faint pink-brown colour. On B.P.B. agar the growth is grey (DAVY's grey), moist and shiny, quite characteristic. The medium after 14 days' growth was acid, pH 3.2

It forms a film and produces acid but no gas in galactose, fructose, lactose and whey. No acid or gas in dextrose (film), mannose (film), arabinose (film), xylose (film), rhamnose, glycerine (film), mannite, sorbite, dulcite, inosite, maltose (film), saccharose (film), raffinose (film), salicin, inulin (film), sol. starch (film), dextrin (film), sodium citrate (film). Does not grow in raisin extract with 5 per cent. alcohol.

In milk there is no apparent change, but a copious yellow sediment, odour yeasty, not clean.

In wort gelatine stick culture the growth is finely beaded to filiform. The surface growth dull and slightly spreading, gradually sinking, liquefying slightly at 37 days. Giant colony in wort gelatine. Cream pink in colour sinking into gelatine, which is softened at 14 days and liquefies slightly at 28 days.

Grows well between 20 and 30° C. Very scant growth at 37° C. Thermal death point 65° for 10 minutes.

Torula ampulla Harrison (Fig. 24).

Cells are large, ellipsoidal, often cuneate, cylindrical or bottle-shaped, ends frequently truncate with rounded corners. Average size 8.5×3.4 μ but much variation. From the wort film 58 days old, the cells are round, ellipsoidal and cylindrical with many irregular shapes. Oil drops in each cell often numerous and vacuoles large. In the sediment, ellipsoidal cells predominate, but long cylindrical, almost mycelial threads are also found.

Budding is from ends or shoulders with often a short and rather thick connecting tubule (Fig. 24).

In wort it forms a creeping film, the liquid at first cloudy, clears, and with age the film becomes somewhat tough.

On wort agar the growth is much raised and slightly verrucous. Whitish in colour. On B.P.B. agar the growth is faint blue in colour, much raised, but in four days it becomes whiter and powdery.

In dextrose and mannose it forms acid but no gas. No gas or acid forms in galactose, fructose, arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, lactose, saccharose, raffinose, salicin, inulin, dextrin and whey. There is film formation in all these sugars accompanied by flocculent turbidity and sediment.

It grows well in raisin extract with 5 per cent. alcohol producing a film.

In milk it produces a thick film, odour very objectionable.

In wort gelatine stick cultures the growth is villous near the surface and filiform below. Surface growth white and powdery. No liquefaction 50 days.

The giant colony is large and white, a verrucous ring near the centre surrounded by a radiately folded margin. No liquefaction—14 days.

Grows well at all tempertaures between 20 and 37° C. Thermal death point 60° C. for 10 minutes.

This organism seems to be somewhat ordium-like, but on account

of its habit of budding, ellipsoidal form, even in the ordium-like films, oil drops, etc. I have classified it with the Torula.

Group C. Slight acidity in dextrose, mannose, galactose or fructose and saccharose -sometimes in lactose.

In this group two, three or four hexoses and saccharose are fermented with the production of acid, and in some instances of small amounts of gas, and consequently of alcohol. In no case does the amount of gas amount to more than one per cent. of the closed tube. A few species produce acid in lactose.

Nearly all the Torulae in this group are round or slightly ellipsoidal. Torulae that have small connecting tubules between mother cell and buds are grouped together.

Torula obvia Harrison (Fig. 25)

This torula and its varieties have been obtained frequently from cheese in many localities. The cells are round from 1.5 μ to 3.2 μ in diameter averaging 2.1 μ. From the wort film and sediment 70 days old cells are round and slightly larger than the young cells.

In wort a ring forms, the liquid is cloudy, but eventually clears, and heavy sediment deposits.

In agar there is a white, waxy raised growth, becoming ridged. On B.P.B. agar the growth is blue, moist, shiny and spreading. pH of medium after 14 days' growth 3.4.

In dextrose, galactose, fructose and saccharose slight acid forms. There is no acid in mannose (film), arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, raffinose, salicin, inulin, dextrin (ring) and whey (ring). In raisin extract with 5 per cent. alcohol there is very little growth. In milk there is no coagulation and no change other than copious sediment. In wort gelatine stick culture the growth is filiform. Surface growth dull and saucer-shape, liquefaction in 50 days. The giant colony is white, with depressed cream-coloured centre.

It grows well at 20-27° C., but very little at 37° C. Thermal death point 10 minutes at 60° C.

Torula obvia -varieties

Var. (*a*). This variety shows the following difference, in all other respects the same characters as above described (Fig. 25):

1. Slight acid in mannose pH 3.2.
2. Slight acid in lactose.
3. Ring forms in raffinose, inulin, whey and citrate.

Var. (b). This variety (Fig. 27) shows the following differences, in other respects the same characters as *T. obvia*:

1. Acid and film in mannose.
2. Ring on xylose.
3. Acid in lactose, a few bubbles of gas in saccharose.
4. Ring in raffinose, growth in citrate.

Var. (c). This variety (Fig. 28) shows the following differences, in other respects the same characters as *T. obvia*:

1. Acid in mannose, film on dextrose, mannose and fructose.
2. Acid and film on lactose, a few bubbles of gas and film in saccharose.
3. Film on raffinose, growth in citrate.

Torula membranacea Harrison (Fig. 29)

The cells are round and sometimes slightly ellipsoidal. They vary from 1.8 to 4.0 μ. Average 3.2 μ. Ellipsoidal cells 3.4×3.0 μ. From the wort film, 58 days old, the cells are very regular in size averaging 3.2 μ. Small 2.0 μ to large 4.5 μ.

In beer wort it produces a creeping film, the liquid, cloudy and sediment, copious. The wort becomes very light in colour.

On wort agar it forms a spreading, dull, whitish growth. On B.P.B. agar a spreading, dull, light blue growth, deeper in colour at the centre and lighter at the margins: pH 4.6.

In dextrose, galactose, fructose and saccharose, it produces slight acidity and a film that is pink coloured from the indicator (andrade).

A creeping film forms but no gas nor acid in mannose, arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, lactose, raffinose, salicin, inulin, dextrine, whey and raisin extract with 5 per cent. alcohol. In milk there is no coagulation, a ring forms.

In wort gelatine stick culture the growth is filiform, the surface growth is flat, dull and spreading, slight liquefaction 60 days. The giant colony is dull, white margin, centre cream-coloured and raised and rounded from centre to edge. It grows well at temperatures between 20-25° C., and very scantily at 37° C. Thermal death point 60° C. for ten minutes.

Torula membranacea—varieties (Fig. 30)

Var. (a) This variety is similar in size and morphology, but has a more vigorous action in some of the sugar broths. Film formation practically identical with the type species.

Differences are:

1. Dextrose, a few bubbles of gas; mannose slight acid formation pH 5.8. Galactose no acid. Saccharose a bubble of gas.
2. Wort gelatine slight liquefaction in 45 days. In all other respects the same characters as *T. membranacea*.

Var. (*b*). This variety differs from *T. membranacea* as follows:

1. Produces acid in mannose, and a faint trace of acid in lactose.
2. Wort gelatine slightly liquefied in 54 days (Fig. 31).
3. Giant colony, soup plate-shaped, centre liquefies in 28 days, the margin frilled and on surface of gelatine.

In other respects the same characters as the type species.

Var. (*c*). This variety differs from *T. membranacea* as follows:

1. Acid in mannose.
2. A few bubbles of gas in saccharose.
3. Scant growth at 37° C.
4. Giant colony different (see Chart and Fig. 32).

Var. (*d*). This variety (Fig. 33) differs from *T. membranacea* as follows:

1. No liquefaction of gelatine 60 days.
2. Slight gas in dextrose, acid in mannose, no acid in galactose.
3. Acid in lactose.

Var. (*e*). This variety (Fig. 34) differs from *T. membranacea* as follows:

1. No liquefaction 60 days.
2. Acid in mannose, no acid in galactose, trace of gas in fructose.
3. Acid in lactose.
4. No film on salicin.

Chemical examination of *T. membranacea*. This was made as before described with the following results:

Total acidity in 500 cc. of a dextrose peptone culture 25 days at 22° C. 15 cc. of normal NaOH for the 500 cc. alcohol present, also traces of aldehyde and acetone. Acetic and lactic acid present, also ethyl acetate. No butyric, formic or proprionic acids.

Torula annulata Harrison (Fig. 35)

Cells round, averaging 3.2 μ in diameter, a few oval cells 3.4 $\times$ 3.0 μ.

The cells from the ring on beer wort after 58 days at 22° C. were round, very even in size averaging 3.2 μ.

In wort it forms a ring, turbid liquid which clears in about 14 days, copious granular sediment.

On wort agar it forms a whitish, shiny spreading growth. With

B.P.B. the growth is cerulean blue in colour and the pH of the medium after two weeks' growth is 3.2.

In dextrose, mannose and fructose it produces a flocculent turbidity, slight acid and a ring. In galactose no acid forms, but there is ring formation.

In arabinose, xylose, rhamnose, mannite, sorbite, lactose, inulin, dextrin, whey, milk and raisin extract a ring forms, but there is no acidity. Slight acid forms in saccharose.

In glycerine, dulcite, inosite, maltose, raffinose and salicin, there is granular turbidity but no ring, acid or gas. No coagulation in milk.

In wort gelatine stick cultures the growth is filiform, surface growth depressed and slight liquefaction in 50 days. The giant colony on wort gelatine is white, dull, smooth and rounded. It grows well at 20 to 25° C., but very scantily at 37° C. Thermal death point 60° C. for ten minutes.

Varieties of *T. annulata* are as follows:

Var. (*a*). This variety (Fig. 36) differs from *T. annulata* in the following particulars, other characters the same:

1. No acid in mannose.
2. No ring in xylose, an islet film and ring on saccharose, a ring on raffinose, no ring on inulin.

Var. (*b*). This variety (Fig. 37) differs from *T. annulata* in the following particulars, other characters the same:

1. Forms ring on wort at 58 days. There are some very large cells 7.0 μ in diameter.
2. Wort agar with B.P.B. pH of medium after two weeks' growth 4.6 .
3. No acid in mannose, no ring on dextrose.
4. No ring on rhamnose, ring on glycerine, film on dulcite, inosite, maltose; no ring on lactose; ring on saccharose and raffinose.

Torula pentosa Harrison (Fig. 38)

Cells are round 1.6 to 3.4 μ in diameter, average 2.8 μ. Slightly ellipsoidal when young and actively growing 3.0×2.5 μ. From beer wort ring, 58 days at 22° C., round cells predominate. They are very regular in size, averaging 3.0 μ in diameter. Cells vary between 2.0 μ and 4.8 μ. There are small tubules between mother cell and buds.

In wort it forms a ring in five days, the liquid at first cloudy and then clearing with a heavy sediment.

In agar there is at first a white spreading growth, which becomes

tinged with a faint Naples yellow colour, this colour in 10 or 12 days browner. On agar with B.P.B. the growth is cerulean blue. After 14 days' growth pH of medium is 4.6.

In dextrose, mannose, galactose and fructose slight acid forms (pH 5.6), as well as a ring.

In arabinose and xylose slight acid forms and a film. In rhamnose, inosite, lactose, raffinose, inulin, dextrin, whey and milk there is ring formation.

In maltose, saccharose there is film formation in both, and acid in the latter.

No film or ring forms on glycerine, mannite, sorbite, dulcite and salicin.

In wort gelatine stick culture the growth is filiform, surface growth dull, slightly raised at first, then gradually sinking into the gelatine, which is slightly liquefied in 54 days. Giant colony is cream-coloured at centre, whitish at margin, smooth, dull and rounded.

It grows well at 20-27, scantily at 37° C., and the thermal death point is 60° C. for ten minutes.

Torula rostrata Harrison (Fig. 39)

Cells are round to ellipsoidal. Round cells average 3.4 μ in diameter, ellipsoidal 3.6 $\mu \times 2.8$ μ to 2.5×2.2 μ. Average 3.4×2.5 μ. From the wort film 58 days' growth at room temperature, the cells are round, from 1.6 μ to 6.0 μ in diameter with an average of 3.4 μ. Buds are frequently attached to mother cell by a small tubule.

In beer wort a thin film forms, the liquid becomes cloudy and remains so for more than seventeen days, then clears.

On wort agar it grows as a spreading, dull growth, the centre thicker and faint yellow in colour, changing to a darker hue with age. With B.P.B. the growth is cerulean blue, moist and shiny at first, then becoming ridged and duller. pH of medium after 14 days' growth 4.6.

In dextrose, mannose, galactose and fructose films form, and slight acid, strongest in dextrose, weakest in galactose. A slight amount of gas about one-half per cent. is present in these hexoses.

In saccharose a film, acid and a half per cent. gas forms.

No gas or acid but film or ring formation is present in the following carbohydrates:

Arabinose, xylose, rhamnose (ring), glycerine, mannite, sorbite, dulcite, inosite, maltose, lactose, raffinose, salicin, inulin, dextrin, whey and raisin extract with 5 per cent. alcohol.

In wort gelatine stick cultures the growth is filiform, surface growth dull and spreading and gradually sinking into the medium.

Slight liquefaction in 54 days. The giant colony in wort gelatine is raised, cream-coloured, dull, with centre slightly depressed.

Grows well at 20-25, not at all at 37° C. Thermal death point 60° C. for ten minutes.

Varieties of *Torula rostrata* (Fig. 40):

Var. (*a*) This variety differs in the following particulars:

1. No gas or acid in galactose. No gas in fructose.
2. No film on dulcite.
3. No liquefaction in wort gelatine stick culture 54 days.

Var. (*b*). This variety (Fig. 41) differs in the following particulars:

1. No gas in the four hexoses. No acid in galactose.
2. No liquefaction in wort gelatine stick culture.

Var. (*c*). This variety (Fig. 42) differs in the following particulars:

1. Striped growth on B.P.B. wort agar.
2. No gas in the four hexoses. No acid in galactose. No film in dextrose.
3. Ring formation instead of film formation on arabinose, xylose, sorbite and whey. No film on lactose.
4. Wort gelatine stick culture, villous. Giant colony slightly different.

Var. (*d*). This variety (Fig. 43) differs in the following particulars:

1. On B.P.B. wort agar pH 3.2.
2. No gas in the four hexoses, no acid in galactose.
3. No films on arabinose, xylose, rhamnose, glycerine, sorbite, dulcite, inosite, salicin.
4. Ring on mannite, maltose, saccharose, raffinose, dextrin and whey.
5. No gas in saccharose.

Group D. Marked acidity and gas in dextrose, mannose, galactose and frustose.

This group has been well represented; numerous isolations have been made from cheese from many districts. Two species predominate.

Torula hexosa Harrison (Fig. 44)

Cells mostly ellipsoidal and regular from wort agar averaging 4.2×2.5 μ. From wort besides the ellipsoidal cells there are some cylindrical 7.5×2.5 μ. From the wort film 50 days at room temperature, the cells are ellipsoidal 6.0×5.0 μ and some round about 6.0 μ in diameter.

In wort there is active fermentation, cloudy at first then clearing. A slight ring forms in seven days which is easily dislodged, but forms again, and is found even after 50 days' growth.

On wort agar it forms a whitish, spreading shiny growth. In wort agar with B.P.B. growth is moist, a faint blue stripe with white margins, the stripe disappears, leaving a pale blue diffused growth. After 14 days' growth pH is 3.2.

In dextrose, mannose, galactose and fructose there is copious gas production and marked acidity. No ring or film develops.

There is no fermentation (no acid or gas) and no film or ring fermentation in arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, lactose, saccharose, raffinose, salicin, inulin, dextrin, whey and citrate. In milk there is no coagulation and no apparent change other than a yeasty odour.

In wort gelatine stick culture the growth is beaded with villous projections from the growth. Surface growth slightly spreading, gradually sinking into gelatine. No liquefaction 58 days. The giant colony is slightly raised, cream-coloured in centre and thicker growth; white around margin and slight radiate markings.

It grows well at temperatures from 20 to 37° C. Thermal death point 60° C. for ten minutes.

Var. (*a*). This variety (Fig. 45) differs from *T. hexosa* in forming slight acid in saccharose, and does not give a ring on beer wort.

Torula rugosa Harrison (Fig. 46)

The cells are ellipsoidal and cylindrical, the former average 5.0×2.5; the latter 8.0×1.6 μ. In milk the cells are smaller, averaging 4.2×2.1 μ. From the wort film after fifty days at room temperature the cells are ellipsoidal from 8.0×4.8 to 4.8×3.2 μ. Cylindrical cells of varying lengths up to 14.0 μ and usually 3.0 μ in diameter.

In beer wort the liquid is turbid, agglutinates and clears in fourteen days; a ring forms, later a film.

On wort agar the growth is whitish, spreading, glistening, quickly becoming rugose. In B.P.B. agar the growth is striped at first with a pale blue centre, later the colour of the central part darkens and the margins change from white to pale blue. The growth is rugose. After 14 days pH is 3.2.

In dextrose, mannose, galactose and fructose there is good gas production and marked acidity.

There is no gas, acid, film or ring formed in arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, lactose,

saccharose, raffinose, salicin, inulin, dextrin, and citrate. In whey a ring forms, and also in milk, but no coagulation; a yeasty odour.

In wort gelatine stick culture the growth is papillate, surface growth umbonate, which sinks into gelatine with age. No liquefaction 60 days. Giant colony in wort gelatine, raised rugose centre, margin folded, pale cream in thicker parts, white in thinner.

It grows well at 20-22° C., and also at 37° C. Thermal death point, 60° C. for 10 minutes.

A chemical examination of a culture of this torula in 500 cc. of dextrose-peptone growth brown at room temperature for 30 days gave the following results: Alcohol, aldehyde and trace of acetone, ethyl acetate, acetic acid; no formic or butyric acid; trace of lactic acid.

Group E. Marked acidity and gas in dextrose, mannose, galactose, fructose and saccharose.

A large number of torula isolated from cheese belong to this group. Morphologically they divide into two divisions: ellipsoidal and round. Film formation on the carbohydrates not common, but occasionally present.

Torula saccharosa Harrison (Fig. 47)

Cells are ellipsoidal, fairly regular in shape and size, rounded ends, budding from ends and shoulders, average size $5.1 \times 2.5\ \mu$.

In wort sediment 70 days at room temperature. The ellipsoidal average $4.0 \times 3.0\ \mu$. A few cylindrical $11.2 \times 3.2\ \mu$ and a few round cells.

In wort there is cloudiness which soon clears. An incomplete ring forms, which soon disappears. The sediment is copious.

In wort agar there is a moist, white, raised shiny growth, which becomes duller and more massive with age, and with edges slightly channelled. With B.P.B. the growth is at first striped, blue down the centre with white margins; later, all growth becomes light blue. The pH of medium at the end of 14 days' growth is 3.2.

In dextrose, mannose, galactose, fructose and saccharose there is marked acidity and plentiful gas. Maximum production occurring about the fourth to the fifth day.

There is no film or ring formation in the above sugars. Good growth but no acid, gas or scums in arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, lactose, salicin, inilin, dextrin, whey or citrate. In milk there is no change, but a yeasty odour.

In wort gelatine stick culture the growth is papillate, the gelatine

much distorted by gas formation, surface growth raised. No liquefaction in 60 days. The giant colony in wort gelatine is white with a scalloped margin and faint concentric contours.

It grows well at 20-25 and also at 37° C. Thermal death point 60° C. for 10 minutes.

The chemical analysis of 500 cc. dextrose-peptone broth gave these results: Alcohol, trace of acetone, trace of aldehyde, acetic and lactic acids. Amyl acetate. No formic, butyric or propionic acid. Total acidity 2.2 cc. normal NaOH per 100 cc. culture.

A number of varieties of this *Torula* were isolated. The most numerous are as follows, the differences only being recorded:

Var. (*a*). Forms a film on wort; growth on B.P.B. wort white pH 4.2. No acid in galactose and giant colony is aborescent (Fig. 48).

" (*b*). Forms a film on the pentoses, arabinose and xylose (Fig. 49).

" (*c*). Moist, white shiny growth without colour on B.P.B. agar. pH 3.4. Acid in whey. More colour (cream) in giant colony (Fig. 50).

" (*d*). White to faint blue in B.P.B. wort agar pH 3.6. Forms a ring on wort (Fig. 51).

" (*e*). Pronounced brownish colour on wort agar, and greenish blue on B.P.B. agar; giant colony crateriform (Fig. 52).

" (*f*). Forms a ring in beer wort. Mottled blue and white on B.P.B. agar. Produces acid in maltose, raffinose, sodium citrate and whey. Giant colony has a cream-coloured centre (Fig. 53).

" (*g*). and " (*h*). These two Torulae are somewhat smaller than *Torula saccharose* and its varieties. Further they are much more irregular in shape and size. The ellipsoidal cells range from 6.5×4.8 μ to 3.2×2.0 μ. Cylindrical cells 8.0×3.0 μ to 4.0×1.5 μ. Their average size is about 4.2×3.0 μ. Their fermentative activity resembles *T. saccharosa* in nearly every detail (Figs. 54 and 55).

Torula dulcis Harrison (Fig. 56)

Cells round from 2.1 to 4.5 μ averaging 3.4 μ. From the wort sediment 60 days at room temperature the cells were round, averaging 3.4 μ.

In beer wort a slight ring and faint film forms which disappears after 40 days. The liquid at first cloudy, soon clears, and a heavy sediment deposits. Odour very fragrant.

On wort agar there is a white, slightly raised, moist, shiny growth,

which later becomes duller and more massive. On B.P.B. agar the growth is white. After two weeks pH is 4.0.

In dextrose, mannose, galactose, fructose and saccharose abundant gas and marked acid forms. There is no film formation.

In arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, lactose, raffinose, salicin, inulin, dextrin, soluble starch, citrate and whey, there is no gas, acid, film or ring formation.

Milk is not coagulated and has a yeasty odour.

Wort gelatine stick culture gives a beaded growth with much gas. No liquefaction in 60 days. The giant colony is a light cream colour, centre flat and margin contoured in concentric zones.

Var. (*a*) differs from *T. dulcis* in the following details:

1. It produces acid in raffinose and inulin.
2. Centre of giant colony is umbonate (Fig. 57).

Var. (*b*) differs from *T. dulcis* in the following details:

1. Agar culture is whitish brown, and B.P.B. agar growth is white to blue-green. pH 3.2.
2. Produces acid in raffinose, acid and a ring in salicin; ring in whey.
3. Gave a ring on wort which persists for 58 days.
4. Giant colony is shaped like a daisy (Fig. 58).

Torula modica Harrison (Fig. 59)

Cells vary in size from 5.4×4.2 μ to 2.5×1.6 μ. Most are ellipsoidal, but round cells also occur in wort and milk.

From the film on beer wort after 50 days' growth at room temperature the cells are round, some slightly ellipsoidal and of varied size from 8.0 to 3.2 with an average of 4.8 μ.

Budding is from any part of the cell, the bud often connected by a short tubule.

In beer wort the liquid becomes very turbid and gassy and lighter in colour. A film forms in five days and persists. Sediment is copious.

In beer wort agar there is a white, raised spreading growth, quickly becoming rugose and dull cream colour. With B.P.B. the growth is white, waxy, ridged or rugose; pH 4.6.

In dextrose, mannose and saccharose there is much gas, marked acidity and film formation.

In galactose gas does not form, but acid and film do. Acid forms also in maltose and whey.

In arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite,

inosite, lactose, raffinose, salicin and inulin, no acid or gas form but film formation in all.

There is no film formation on glycerine.

In wort gelatine stick culture the growth is villous to beaded, with secondary villous tufts. In 35 days the gelatine begins to liquefy and complete liquefaction follows in 45 days.

The giant colony is large and white, rugose and folded from centre to edge, sinking into gelatine and eventually liquefying it.

Grows well at 20-22 and very well at 37° C. Thermal death point 60° C. for 10 minutes.

Torula arta Harrison (Fig. 60)

Cells ellipsoidal, cylindrical, often with one end pointed. The general appearance of young cells from beer wort and wort agar is that of narrow cells. Dimensions from 5.1×1.6 to 3.8×0.9 μ. From the film in wort after 50 days' growth at room temperature, the cells are ellipsoidal and round; the former 3.0×2.6 μ, the latter from 3.2 to 1.8 μ in diameter. Budding from ends, shoulders and sides.

In beer wort a ring and afterward a veil-like film forms; the liquid is turbid and sediment heavy.

On wort agar it grows as a white spreading mass, with edge contoured, shiny at first and then duller.

With B.P.B. the growth is white and shiny, then faint blue and after seven days whitish; pH 4.6.

In dextrose, mannose, galactose, fructose and saccharose it forms abundant gas, acid and films. In maltose and dextrin no gas, but acid and a film.

There is film formation in glycerine, mannite and citrate and a ring on lactose and whey.

On arabinose, xylose, rhamnose, sorbite, dulcite, inosite, lactose, raffinose, salicin, inulin, no gas, acid, film, nor ring.

In milk there is no coagulation, but slight alkalinity and an unpleasant odour.

Wort gelatine stick culture the growth is beaded, with villous tufts in upper third, surface growth frilled. No liquefaction in 60 days. The giant colony is porcelain white, flat and spreading, edges lobate and scalloped, concentrically contoured rings and radiate markings.

Grows well at 20 to 22, and luxuriantly at 37° C. Thermal death point 60° C. for 10 minutes.

Group F. This group produce marked acidity and gas in dextrose, mannose, galactose, fructose, saccharose and raffinose.

Commonly occurring in many cheese from widely separated factories in Ontario and Quebec.

Torula raffinosa Harrison (Fig. 61)

Cells round, when actively growing somewhat ellipsoidal, budding from any part of the cell.

Size varies from 3.6 to 1.6 μ. Average 2.8 μ. From the sediment in wort 58 days at room temperature, the cells vary from 4.0 μ to 1.6 in diameter, the majority very regular 2.8 μ. Some oval cells 2.8$\times$ 2.3 μ.

Beer wort is at first cloudy, clears about the sixth day and remains clear. No film or ring forms (70 days); sediment heavy.

On wort agar there is an abundant, white, spreading growth, ridged in the centre and of a faint brown colour, where the growth is thickest.

With B.P.B. there is a blue-green stripe with white margin, turning later to light blue; pH 3.6.

In dextrose, mannose, galactose, fructose, saccharose and raffinose there is marked acidity and gas, but no film nor ring.

In arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, lactose, salicin, inulin and dextrin there is no gas, acid, film nor ring, but good growth at first cloudy and then clearing with more or less sediment. In milk there is weak acid, no coagulation and yeasty odour.

In wort gelatine stick culture the growth is filiform to beaded. Surface growth gradually sinks into gelatine. No liquefaction in 74 days. The giant colony is white and dull, centre slightly raised, with faint, zonal concentric markings to the edge.

Grows well at 20-25, scantily or not at all at 37° C. Thermal death point 60° C. for 10 minutes.

This Torula has been isolated many times and seems to be one of the commonest varieties in cheese.

The chemical products of growth (from the examination of 500 cc. dextrose-peptone culture of this organism grown for 25 days at room temperature) were as follows: alcohol, aldehyde and trace of acetone; ethyl acetate, acetic and lactic acids; no formic acid.

There are a number of varieties of this organism; the more common ones are mentioned below.

Varieties of T. raffinosa:

Only differences from the type species recorded; characteristics not mentioned the same.

Var. (*a*) of *T. raffinosa* (Fig. 62):

1. pH in B.P.B. agar 4.0; white growth.
2. Produces acid and a ring, but no gas in dextrin.

Var. (*b*) produces acid, but no gas or film in inulin (quite common) (Fig. 63).

Var. (*c*) of *T. raffinosa* (Fig. 64):

1. Dark cerulean blue; pH 3.2 in B.P.B. wort agar.
2. Liquefies slightly wort gelatine in 60 days.
3. Forms a ring on whey.
4. Giant colony, shallow crateriform.

Group G. This group produces marked acidity and gas in dextrose, mannose, galactose, fructose, saccharose and maltose.

Torula maltose Harrison (Fig. 65).

The young cells from wort are ellipsoidal and occasionally round. From wort agar the cells are ellipsoidal, and cylindrical, long threads (mycelial formation) are present. Size varies from 7.0×3.0 μ to 3.0×1.5 μ, the average about 6.0×3.4 μ. From the wort film fifty days old the cells are round, ellipsoidal and cylindrical with mycelial threads. The majority of cells average 4.8 μ in diameter, many with thick cell walls. The mycelial threads average 2 μ in width and varying lengths.

In beer wort there is active fermentation, the liquid becomes cloudy, a film forms on the fourth day. Film becomes thicker with age, and is somewhat tenacious.

On wort agar the growth is dull, raised and thick, becoming rugose and light cream colour. On B.P.B. agar the growth is white and waxy; medium becomes more alkaline.

In dextrose, mannose, galactose, fructose, maltose, and saccharose it forms gas, acid and films.

In arabinose, xylose, rhamnose, sorbite, inosite, raffinose, salicin, inulin, dextrin, whey and citrate there is flocculent turbidity which subsequently clears, and film formation.

In wort gelatine stick culture the growth is beaded and slightly villous in the upper half. Surface growth sinks and liquefaction begins about the fortieth day and progresses slowly until the gelatine is all liquefied. The giant colony is raised, centre verrucous to vermiform, the margin concentrically contoured with radiate markings and frilled edge.

It grows luxuriantly at 37 and well at 20 to 25° C. Thermal death point 60° C. for ten minutes.

A culture in 500 cc. dextrose-peptone broth after 30 days' growth, when examined by Chester's method, gave the following results: alcohol, acetone, acetic and lactic acid. Esters also present, probably ethyl and amyl acetate.

In groups *II* and *I* are included the lactose fermenting yeasts; in the former group those that ferment lactose, and in the latter inulin as well as lactose.

Group II. This group produces marked acidity and gas in dextrose, mannose, galactose, fructose, saccharose and lactose.

The group of lactose fermenting organisms have been very frequently found in cheese. They produce, as a rule, esters and similar aromatic compounds. Their odour, when growing in pure culture, is usually agreeable, frequently fruity, which, of course, is not desired in cheese.

Torula lactosa Harrison (Fig. 69)

Cells usually ellipsoidal to round. In young cultures the cells are mainly ellipsoidal, averaging 3.4×3.0 μ; round cells 3.2 μ in diameter. From the wort film fifty days at room temperature, the cells are round and ellipsoidal, the former from 4.8 to 3.2 μ, averaging 3.6 μ; the latter about 4.0×3.2 μ.

In beer wort the growth is cloudy, a ring and islet film forms which is easily disturbed. The liquid clears with a copious sediment.

On wort agar the growth is white, raised, spreading and shiny, becoming more massive and dull with age. On agar with B.P.B. the growth at first white becomes light blue-green with a pH of the medium of 4.2.

In dextrose, mannose, galactose, fructose, saccharose, lactose and whey, there is much gas, marked acidity and film formation.

In arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, salicin and inulin, there is no acidity, film nor ring formation.

A film forms but there is no acid nor gas in maltose, raffinose nor dextrin.

In milk much gas forms, and there is slight acidity and a copious sediment. No coagulation (50 days) odour yeasty and aromatic.

Wort gelatine stick culture, growth papillate, surface growth at first umbilicate then sinks into gelatine; slight liquefaction 60 days. The giant colony is saucer-shaped, frilled margin, depressed centre and slight liquefaction (28 days).

It grows well at 37 and also at 20-25° C. Thermal death point 60° C. for ten minutes.

Torula spumosa Harrison (Fig. 70)

Cells ellipsoidal and cylindrical, rounded ends, sometimes one end slightly pointed. Ellipsoidal cells average 4.5×3.0 μ; cylindrical 7.0×2.5 μ. From the wort ring after fifty days' growth the cells are mostly ellipsoidal from 6.0×3.2 μ to 4.8 to 3.2 μ.

In wort it produces a turbid, foaming fermentation, reducing the colour and forming a ring. The liquid clears in seven days and remains limpid.

In wort agar the growth is white, raised, spreading and shiny, becomes thicker in the centre of the slope and then duller and massive. In wort agar with B.P.B. the growth is white. After 14 days' growth pH of medium is 3.4.

In dextrose, mannose, galactose, fructose, saccharose, lactose and whey it produces much gas and marked acidity, except that in galactose no gas forms. There is no ring nor film formation.

In arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, maltose, raffinose, salicin, inulin, dextrin and citrate, no gas, acid nor film forms. The growth is poor in the carbohydrate alcohols and in raffinose.

In milk it produces much gas, which foams almost to the top of the tube when shaken. Weak acid forms, and a yellowish sediment, but there is no coagulation; the odour is yeasty and bitter.

In wort gelatine stick culture the growth is papillate to beaded; upper portion of the stick has villous tufts projecting from the beaded growth. Surface growth sinks into the gelatine. Slight liquefaction, but incomplete 60 days. The giant colony is large, flat spreading with a pink tinge at margin. Zonal concentric contours in central area.

It grows well at 20-22 and also at 37° C. Thermal death point 62° C. for 10 minutes.

Torula lactophila Harrison (Fig. 71)

Cells round or slightly ellipsoidal from 4.0 to 1.8 μ in diameter, averaging 3.4 μ. The ellipsoidal cells 3.4×3.0 μ. From the wort film after 58 days' growth at room temperature the cells are round, 3.4 μ in diameter and some ellipsoidal 3.8×3.0 μ. Smaller cells are more frequent than large ones.

In beer wort there is granular cloudiness, much gas, and a ring forms on the sixth day, becoming heavier with age. An islet film develops on the eleventh day and persists, reforming if shaken down.

On wort agar the growth is white, moist, raised, higher in the centre; colour becomes a faint Naples yellow.

With agar and B.P.B. the growth at first white becomes a greenish-blue tinge. After fourteen days' growth pH of medium is 3.2.

In dextrose, mannose, fructose, lactose, saccharose and whey it produces acid and gas.

In galactose acid but no gas (repeated tests).

Film formation present in galactose, fructose, lactose and saccharose.

In arabinose, xylose, rhamnose, glycerine (ring), mannite, sorbite, dulcite, inosite, and dextrin, no gas, acid, film nor ring formations.

Film formation present on maltose, raffinose (also acid pH 5.2) and inulin, and a ring on salicin.

In milk there is abundant gas formation, weak acid, a slight ring, but no coagulation; the odour is yeasty and slightly bitter.

Wort gelatine stick culture. The growth is papillate to filiform. Surface growth flat and depressed. No liquefaction in 60 days. The giant colony has a central area faint yellow cream in colour surrounded by a white margin with radiate markings. Grows well at 20-25 and also at 37° C. Thermal death point 62° C. for 10 minutes.

Group I. This group produces marked acidity and gas in dextrose, mannose, galactose, fructose, saccharose, lactose and inulin.

Members of this group have been isolated from over twenty cheese coming from different localities. The fermentative activity of all members of the group are very similar, but the morphological picture varies considerably. All produce ester-like odours.

Torula inulacea Harrison (Fig. 72)

Young cells from wort are ellipsoidal and fairly regular, averaging 4.2×2.5 μ. From wort agar the cells vary, besides ellipsoidal there are many cylindrical, up to 12.0 μ long×2.1 μ wide. From sediment in wort after fifty days' growth the cells are larger, ellipsoidal 6.0×4.0 μ, round 4.8 μ, and cylindrical 6.0×2.0 μ.

In beer wort turbidity is quite marked, soon clearing; a slight ring forms but disappears, and cultures at fifty days old have no ring nor film, are quite clear, with heavy sediment.

On wort agar the growth is white, raised, spreading, glistening, the centre of the slope higher, giving a ridged appearance. With B.P.B. added the growth is still white. The pH of medium after 14 days' growth 4.6.

In dextrose, mannose, galactose, fructose, lactose, saccharose, inulin and whey, there is marked acidity and gas but no film nor ring formation.

On arabinose, xylose, rhamnose, glycerine, mannite, sorbite,

dulcite, inosite, maltose, raffinose, salicin, dextrin, and citrate there is growth but no acid, gas, film nor ring formation.

In milk there is slight acidity and much gas but no coagulation.

In wort gelatine stick culture the growth is beaded and becomes hazy around the upper growtn; the gelatine liquefies slowly, beginning about the 30th day and is complete in 58 days. The giant colony is dull, flat with slightly raised centre, brownish white in colour, the margin white and radiately contoured. The giant colony is dull, flat with slightly raised centre brownish-white in colour, the margin white and radiately contoured.

It grows luxuriantly at 37° C., and well at temperatures between 20 and 25° C.

Thermal death point 62° C. for 10 minutes.

Variety of *T. inulacea*.

The following variety (Fig. 73) has been isolated, and differs in the following details, all other reactions similar to *T. inulacea:*

Var. (*a*). 1. Forms a ring on wort.
2. Does not liquefy gelatine (64 days).
3. Produces acid in raffinose, pH 6.2.
4. Giant colony, white, with three zonal concentric contours and radiate markings.

The chemical products of growth, from 500 cc. of a dextrose-peptone culture, after 28 days' growth, were: alcohol, acetone, esters (ethyl acetate, ethyl butyrate), acetic acid, butyric acid (trace) and lactic acid.

Torula aromatica Harrison (Fig. 74)

Cells ellipsoidal and cylindrical, very irregular in size and shape, ellipsoidal cells with rounded ends, or one end rounded and the other somewhat pointed. Size of ellipsoidal cells from 7.0×3.5 μ to 3.0×1.5 μ, the majority 5.5×2.8 μ. Cylindrical cells all lengths 9.0 to 2.0 μ×1.6 μ.

From the ring on wort 58 days old the cells are ellipsoidal and cylindrical; ellipsoidal 5.0×3.4 μ to 4.2×2.5, cylindrical 8.5×2.5 μ.

In wort there is marked turbidity and a film forms; also a marked ring. The liquid clears and becomes lighter in colour.

On wort agar there is a spreading, white growth, and a white growth on B.P.B. agar. After 14 days' growth pH of medium is 3.2.

In dextrose, mannose, galactose, fructose, lactose, saccharose, inulin, and whey, there is marked acidity and gas but no film nor ring formation. In raffinose acid but no gas.

In arabinose, xylose, rhamnose, glycerine, mannite, sorbite, dulcite, inosite, maltose, salicin, and dextrin there is no acid, gas, film nor ring.

In milk, acid and much gas form. The milk has a distinct fruity odour.

In wort gelatine stick culture the growth is filiform, surface growth waxy, gradually sinking into gelatine, which commences to liquefy the 20th day and progresses slowly, is stratiform in shape, and incomplete in 60 days. The giant colony on wort gelatine is dull, flat with a cream-coloured, raised, concentric ring near the centre, and from there to margin fine radiate markings.

Grows luxuriantly at 37 and well at 20-25° C. Thermal death point 60° C. for 10 minutes.

The chemical products of growth were alcohol, esters (ethyl acetate) and acetic, lactic and proprionic acids.

Varieties of *T. aromatica:*

These varieties differ in the following details from *T. aromatica:*

Var. (*a*). 1. Rugose growth on wort agar; pH of media B.P.B. 4.0.
2. No acid in raffinose (Fig. 75).

" (*b*). 1. Finely rugose on wort agar. Pale blue on B.P.B. wort agar. After growth pH of medium is 3.4.
2. Does not liquefy gelatine (52 days).
3. Giant colony brownish white (Fig. 76).

" (*c*). 1. Forms a film on galactose, ring on fructose and inulin.
2. Giant colony, warty centre, irregular lobate edge (Fig. 77).

Viability

The longevity of yeasts under different conditions is remarkable. DUCLAUX (37) found some of PASTEUR's beer yeasts alive after 22 to 23 years. KLOCKER has found living yeast cells in beer wort solution after 20 and 30 years. HARRISON noted the presence of yeast cells (Torulae) in cheese a year old.

In the present investigation a number of cheese plugs have been kept for 180 days at a temperature of 10° C., although badly moulded numerous yeast cells were present; one sample gave over 250,000 cells to the gram. In the oldest cheese I could find in Montreal, two year and six months old, yeast cells were present in small numbers. This cheese had been selected for its fine flavour when about six months old. Cultures 200 days old quite dried out often contain living cells.

Hence, it is evident that yeasts, including torula, remain alive for long periods, and cheese containing large numbers of torula when made retain living cells, frequently in large numbers, for periods up to

two-and-a-half years old. My previous investigation has also shown that there is an actual increase of torulae in cheese after it is made.

Flavour in Milk

All the organisms described have been cultured repeatedly in milk, either as pure cultures or associated with *S. lactlactis*. The odour or flavour of all was examined by four experienced graders belonging to the staff of the Division of Dairy Products of the Dairy and Cold Storage Branch of the Dominion Department of Agriculture. Their remarks in many instances have been incorporated with the description of the Torulae isolated from cheese under the heading of milk. The majority of the cultures were called "yeasty" by the graders, but in addition such remarks as "not clean," "very objectionable," "bitter yeast," "fruity," "very unclean," "whey tank," "surface taint," occur with considerable frequency.

When associated with the lactic acid bacteria, the flavour is often completely changed but it cannot be said to be improved. Esters have been identified from some of the cultures, and the detection of strawberry, pineapple and pear flavours gives probability to the presence of ethyl acetate, ethyl butyrate, amyl acetate, ethyl formate, etc.

The presence of lactic, formic, acetic, propionic, butyric, caproic and succinic acids, ethyl alcohol, and esters made up of compounds of ethyl alcohol, acetic, caproic and butyric acids, and probably succinic acid in normal cheddar cheese, have been demonstrated by SUZUKI, HASTINGS and HART (38), and in all probability the presence of yeasts yielding alcohol and other compounds favours the production of many more esters.

The cultures examined chemically were grown in broth containing much less nitrogen and sugar than is the case with cheese, and hence experiments are planned in order to ascertain the presence of various esters in cheese made with cultures of some of the yeasts here described.

It is highly probable that ethyl butyrate, ethyl acetate, ethyl formate, and other esters are formed in sufficient quantity, through the agency of yeasts to produce the odours detected in fruity and other flavours. Aldehyde, too, may be the cause of some of the objectionable flavours.

Habitat

The yeasts described in this paper have been isolated from cheese. Investigation is now proceeding in order to find out how these yeasts gain access to the milk, and this data will be correlated with the present investigation at a later date.

REFERENCES

(1) PIROTTA & RIBONI, 1879, Rendiconti d. R. Inst. Lombard.
(2) DUCLAUX, E., 1887, Ann. de l'Inst. Past., I, 573.
1889, Revue critique, *Ibid.*, 201.
(3) BEIJERINCK, M. W., 1889, Arch. neerlandaises des Sci. exactes et nat., **23**, 428.
(4) BOCHICCHIO, N., 1894, C'bl. f. Bakt., **11**, 546.
Ann. de Micro., **6**, 165.
(5) HARRISON, F. C., 1900-1, Trans. Can. Inst., **7**, 129.
1902, Bitter Milk & Cheese, C'bl. f. Bakt. II, **9**, 206. Ont. Dept. of Agr., Toronto, Bull. **120**
(6) HARDING, H. H., 1900, New York Expt. Sta. Bull., **183**.
(7) HASTINGS, E. G., 1905-1906, Wisconsin Expt. Sta. Ann. Rept., **22** and **23**.
(8) EDWARDS, S. F., 1913, C'bl. f. Bakt. II, Abt. **39**, 449.
(9) CORDES, W. A. and HAMMER, B. W., 1926, 1927, Jour. Dairy Sci., **9**, 508; **10**, 50.
(10) GUILLIERMOND, A., 1920 (trans. by F. W. TANNER), The Yeasts.
(11) WILL, H., 1903, 1907, C'bl. f. Bakt., Abt. II, **10**, 689; **17**.
(12) LEBERLE & WILL, 1907-8, *Ibid.*, **21**.
(13) LUTZ, L. & GUEGEN, 1901, Bull. Soc. Myc. de France, **17**, 83.
(14) HUCKER, G. J., 1921, Tech. Bull. **87**, New York Exp. Sta.
(15) SCARTH, G. W., Plant Physiology, Vol. **1**, 215.
(16) KLOCKER, 1906, Die Gärungsorganismen, 292.
(17) PASTEUR, L., 1876, Etudes s. la bière, 73.
(18) HANSEN, E. C., 1883, C. R. des trav. lab. de Carlsberg, II, **2**.
(19) FRESENIUS, 1850-1863, Beit. z. Myk.
(20) COHN, F. & SCHRÖTER, J., 1872, Beit. z. Biol. der Pflanzen, I.
(21) HANSEN, E. C., 1879, C. R. des trav. lab. de Carlsberg, **1**, Book 24.
(22) PRINGSHEIM, F. & BILEWSKY, H., 1910, Beit. z. Biol. d. Pflanzen, **10**.
(23) WILL, H., Handbuch der Technischen Mykologie, **4**, 280.
(24) LINDNER, P., 1887, Wochenschift f. Brau., **4**, quoted from Guilliermond.
(25) GROSBUSCH, T., 1915, C'bl. f. Bakt., II, **42**, 625-638.
(26) JORGENSEN, A., 1909, Die Mikroorganismen der Gärungsindustrie.
(27) VAN HEST, J. J., 1903, Zeit. f. d. ges. Brauwesen, **26**, 808.
(28) SARTORY, A., 1907, Bull. Soc. Myc. de France, **23**.
(29) KITA, V. G., 1912, Orig. communication 8th Internat. Congress of App. Chem., **14**, 99-106.
(30) KALANTHAR, A., 1898, Zeit. f. physiolog. Chem., **26**, 88.
(31) MARPMANN, G., 1886, C'bl. f. allgem. Gesunheitspflege, 422.
(32) WILL, H. and NOLDIN, F., Zeit. f. ges. Brau, **37**, 13-16.
(33) CHESTER, F. D., 1901, Manual of Determinative Bacteriology, 37.
(34) ALLEN, H. H., 1898, Commercial organic analysis, Third Ed., **1**.
(35) ADAMETZ, L., 1899, C'bl. f. Bakt. **5**.
(36) KAYSER, E., 1891, Ann. de l'Inst. Pasteur, **5**.
(37) DUCLAUX, E., 1898, Traité de Microbiologie, **43**.
1895, Ann. de l'Inst. Pasteur, **9**, 266.
(38) SUZUKI, S. K., HASTINGS, G. G. and HART, E. B., 1910, Res. Bull., **11**, Ws-i consin Agr. Expt. Sta.

Illustrations

All photomicrographs (except 1, 2, 3, 4, and 5) were made from preparations obtained from beerwort cultures, 36 hours old at 22° C., stained by Gram's method; 1 and 2 were made direct from cheese, stained by Gram's method, counterstained with Safranin; 4 and 5 were made from plaster block preparations, fixed in methyl-alcohol, and stained by Jenner's stain, followed by dilute Giemsa, the excess stain washed out in water containing a trace of acetic acid.

(Original photomicrographs were ×600, reduced in reproduction to approximately ×370.)

1. Yeast colony in cheese. A few bacteria may be noted.
2. Yeast colony in cheese. A few bacteria may be noted.
3. *Saccharomyces casei.*
4. *S. casei,* showing spores.
5. *S. casei,* showing spores beginning to germinate.
   Note. Cell walls of the ases.
6. *Rhodotorula corralina.*
7. *Chromotorula ochracea.*
8. *Torula dubia.*
9. " *Var. a*
10. " " *b*
11. " " *c*
12. " " *d*
13. " " *e*
14. *T. subacida.*
15. *T. acidula.*
16. " *Var. a*
17. " " *b*
18. " " *c*
19. " " *d*
20. " " *e*
21. " " *f*
22. *T. angusta.*
23. *T. rostella.*
24. *T. ampulla.*
25. *T. obvia.* *Var. a*
27. " " *b*
28. " " *c*
29. *T. membranacea.*
30. " *Var. a*
31. " " *b*
32. " " *c*
33. " " *d*
34. " " *e*
35. *T. annulata.*
36. " *Var. a*
37. " " *b*
38. *T. pentosa.*
39. *T. rostrata.*
40. " *Var. a*
41. " " *b*
42. " " *c*
43. " " *d*
44. *T. hexosa.*
45. " *Var. a*
46. *T. rugosa.*
47. *T. saccharosa*
48. " *Var. a*
49. " " *b*
50. " " *c*
51. " " *d*
52. " " *e*
53. " " *f*
54. " " *g*
55. " " *h*
56. *T. dulcis.*
57. " *Var. a*
58. " " *b*
59. *T. modica.*
60. *T. arta.*
61. *T. raffinosa.*
62. " *Var. a*
63. " " *b*
64. " " *c*
65. *T. maltosa.*
69. *T. lactosa.*
70. *T. spumosa.*
71. *T. lactophila.*
72. *T. inulacea.*
73. " *Var. a*
74. *T. aromatica.*
75. " *Var. a*
76. " " *b*
77. " " *c*

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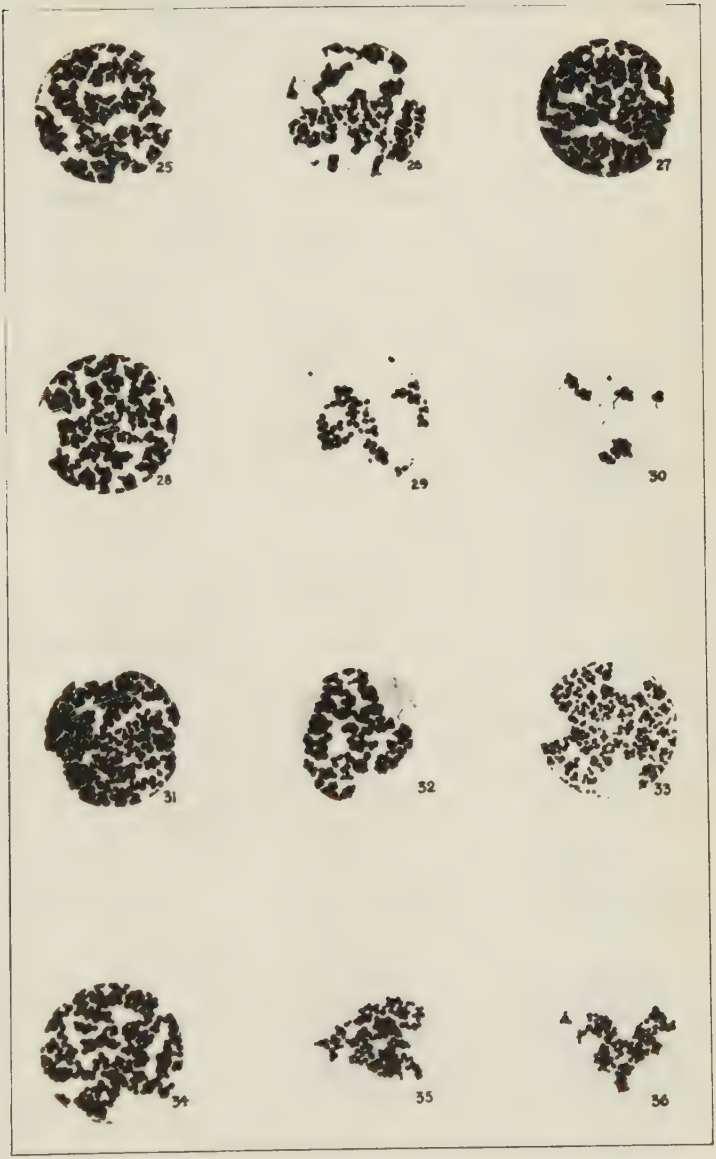
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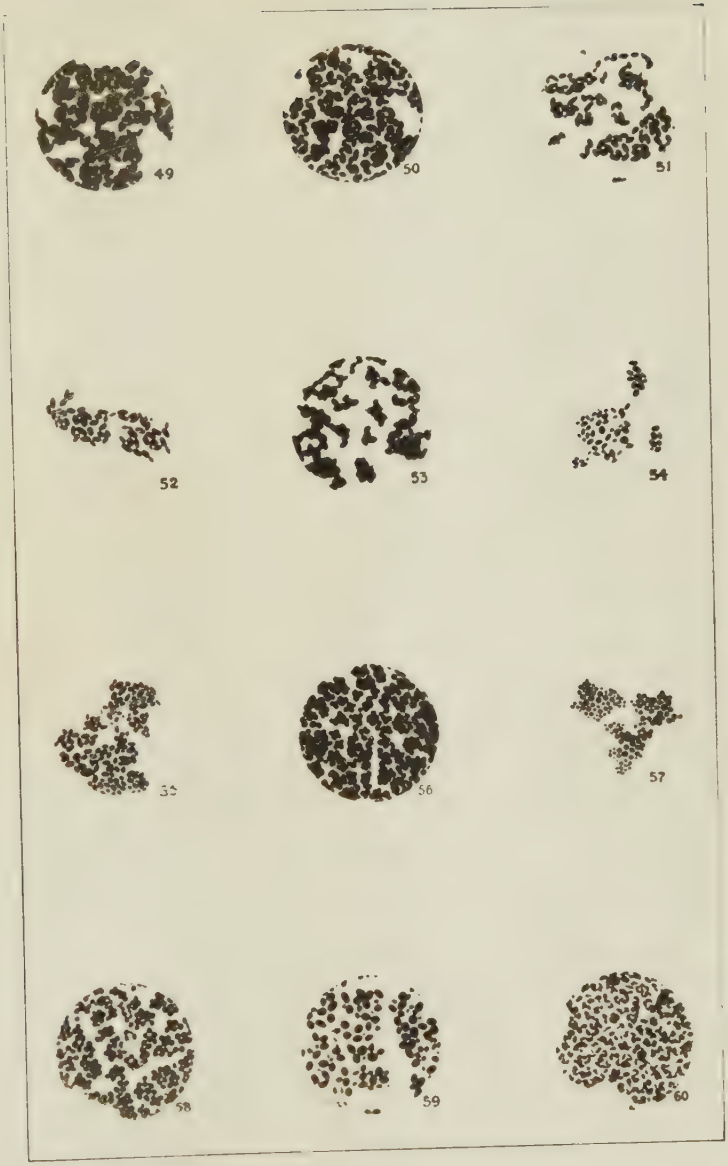
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